CLAIMS

We claim:

1. A method of measuring cell death or membrane damage in a mixture of dead and living cells or in a supernate therefrom, comprising determining in a sample of a mixture of dead and living cells or a supernate from the mixture the concentration of a high-energy molecule, such as NADH, NADPH, or ATP, by a luminescent reaction employing a luciferase, wherein an enzyme or enzymes is naturally present in the living cells being studied, and, when released from the dead cells, increases or decreases the concentration of the high-energy molecule by a reaction or reactions, whereby all the reactions necessary to produce the light output are initiated when the sample is contacted with a single reagent mixture.

2. The method of claim 1, wherein the enzyme or enzymes released is/are present in all known living cells.

3. The method of claim 2, wherein activity of the enzyme or enzymes released from the dead cells is coupled to that of one or more additional enzymes, such that a reaction product or products of said released enzyme or enzymes is a reaction substrate or substrates of one or more of said additional enzyme or enzymes, whereby said additional enzyme or enzymes increase or decrease the concentration of the high-energy molecule.

4. The method of claim 3, wherein the luciferase is firefly luciferase, the enzyme released from the dead cells is glyceraldehyde-3-phosphate dehydrogenase, and the activity of the enzyme released is coupled to that of the additional enzyme phosphoglycerokinase to produce the high-energy molecule ATP.

5. The method of claim 4, wherein the sample is treated to convert living cells to dead cells prior to, simultaneously with, or after contact with the single reagent mixture, and the luminance signal generated thereby is read at any point or points in the

process subsequent to addition of the reagent mixture, which may be before, after, or both before and after the conversion of live cells to dead cells.

6. The method of claim 4, wherein a potentially cytotoxic agent is added to the sample, prior to or simultaneous with contact with the single reagent mixture.

7. The method of claim 5, wherein the cells to be measured for cytotoxicity, killed, and measured again for total biomass, are nucleated eukaryotic cells.

8. The method of claim 5, wherein the cells are killed by addition of a detergent which has either negligible or predictable and reproducible effects on the performance of the cytotoxicity assay.

9. The method of claim 8, wherein the detergent is Nonidet P-40, NP-40, or Brij.

10. The method of claim 5, wherein the cells to be measured for cytotoxicity, killed, and measured again for total biomass, are Gram-negative bacteria.

11. The method of claim 10, wherein the cells are killed by a mixture of a pore-forming agent and an enzyme which digests the cell wall.

12. The method of claim 11 wherein the pore-forming agent is Polymyxin B and the digestive enzyme is lysozyme.

13. The method of claim 5 wherein the cells to be measured for cytotoxicity, killed, and measured again for total biomass, are Gram-positive bacteria.

14. A method of measuring or monitoring the concentration of free inorganic phosphate in a sample, comprising contacting the sample with glyceralde-3-

phosphate dehydrogenase, phosphoglycerokinase, and luciferase in a coupled reaction system, together with appropriate buffer constituents, cofactors, and substrates for the respective enzymes, such that luminance emitted by the system is related to the concentration of free phosphate.

15. The method of claim 14 wherein the buffers and other constituents used, apart from the phosphate source being tested, are wholly or substantially free of phosphate.

16. The method of claim 14 wherein the free phosphate concentration thereby measured or monitored indicates the activity of a phosphatase.

17. The method of claim 16, wherein the phosphatase is a protein phosphatase.

18. The method of claim 17, wherein the phosphatase is a protein tyrosine phosphatase.

19. The method of claim 16, wherein the activity of the phosphatase thereby measured or monitored identifies inhibitors or other modulators of the activity of the phosphatase.

20. The method of claim 16, wherein the phosphatase activity takes place under conditions that are incompatible with the light reaction described, but wherein the phosphatase is contacted by one or more substrates in a first step, whereupon in a second step the conditions are adjusted appropriately for operation of the light reaction described and the light reaction is used to detect free phosphate.